Serum cytokine profiles distinguish acute food protein-induced enterocolitis syndrome from mimicking diseases

Tamaki Nakamura[1], Seigo Okada[1], HIROKI YASUDO[1], Ken Fukuda[2], Fumiko Okazaki[2], Hiroyuki Wakiguchi[1], Yuno Korenaga[1], Seigo Korematsu[3], Kenji Matsumoro[4], Shoichi Ohga[5], and Shunji Hasegawa[1]

[1]Yamaguchi University Graduate School of Medicine
[2]Yamaguchi University School of Medicine Graduate School of Medicine
[3]Oita University Faculty of Medicine
[4]National Research Institute for Child Health and Development
[5]Kyushu University

September 24, 2020

Abstract

Background: Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE cell-mediated food allergy characterized by repetitive vomiting and other gastrointestinal symptoms. Although little is known about FPIES pathophysiology, some cytokines have been reported to be involved. Since one of the main symptoms is vomiting, which is common to other diseases, it is difficult to distinguish acute FPIES from other conditions such as infectious enterocolitis. Thus, specific biomarkers are required for differential diagnosis. We aimed to identify potential biomarkers distinguishing acute FPIES from infectious enterocolitis and IgE-mediated anaphylaxis, which also cause vomiting. Methods: Seven patients with acute FPIES, nine with IgE-mediated anaphylaxis, and six with infectious enterocolitis were enrolled. The serum concentrations of interleukins (IL)-2, -4, -6, -8, -10, interferon-γ, and tumor necrosis factor-α were measured and compared among the three groups of patients. The serum concentrations of IL-2 and IL-10 were also compared between the symptomatic and asymptomatic stages. Alterations in serum cytokine levels were evaluated in acute FPIES during an oral food challenge test. Results: Serum IL-2 and IL-10 levels were significantly higher in acute FPIES patients than in patients with infectious enterocolitis and IgE-mediated anaphylaxis, whereas no significant differences were detectable in the serum levels of the other cytokines. The IL-2 and IL-10 elevation was only observed in the symptomatic stage of acute FPIES. Conclusion: The elevation in serum levels of IL-2 and IL-10 was specifically observed in symptomatic acute FPIES cases, suggesting that the measurement of IL-2 and IL-10 could be employed for differential diagnosis.

Introduction

Food protein-induced enterocolitis syndrome (FPIES) is a non-IgE cell-mediated allergy characterized by repetitive vomiting and diarrhea after ingestion of offending food. Symptoms are often severe and can result in acute dehydration and lethargy.[1] Although FPIES cases are currently increasing, little is known about the pathophysiology of this disease. In the absence of specific laboratory tests, diagnosis predominantly relies on clinical responses to elimination diets with a resolution of symptoms, oral food challenges with reappearance of symptoms following ingestion of the offending food, results of endoscopic and biotic exams, and exclusion of causes such as infection, inflammatory bowel disease, ischemia, and metabolic disorders.[2]

Previous studies have suggested that several cytokines are involved in the pathogenesis of FPIES.[3-5] The serum levels of interleukin (IL)-2 and IL-10 were found to be elevated in patients with FPIES after oral food challenge (OFC) tests.[4,5]Kimura et al.[4]reported that the increase in T cell-produced serum IL-2, detected

in OFC-positive patients, is associated with FPIES pathophysiology. Caubet et al.[5] suggested that, in patients with acute FPIES, T cells have a reduced capacity to secrete IL-10, and other cell populations, such as monocytes, may produce compensatory IL-10 in response to antigen exposure. The increased serum level of IL-8 and the elevation of neutrophils after OFC suggest that neutrophils play an important role in acute-phase FPIES.

This disease is classified as acute or chronic FPIES and each timing and duration of symptoms is different. Recent international consensus guidelines define acute FPIES by the following criteria: 1) it occurs after intermittent food exposure, 2) emesis usually starts within 1–4 hours and is accompanied by lethargy and pallor, 3) diarrhea can follow within 24 hours (usual onset after 5–10 hours), and 4) symptoms usually resolve within 24 hours after elimination of the food from the diet.[6] However, due to the absence of predictive biomarkers, these criteria are mainly based on clinical symptoms.

Since the main symptom of acute FPIES is vomiting, analytical tools capable of distinguishing FPIES from mimicking diseases that also cause vomiting, such as infectious enterocolitis, are strongly required. Lee et al.[7]examined the clinical and laboratory characteristics of patients referred to emergency departments for symptoms suggestive of acute FPIES, such as lethargy, floppiness, pallor, and normal C-reactive protein (CRP), and compared these features with those of gastroenteritis and bacterial sepsis. However, their retrospective study had substantial limitations because the diagnoses were not made by allergists, and comprehensive data, including FPIES-related cytokines, were not evaluated.

Therefore, we aimed to identify biomarkers capable of differentiating acute FPIES from infectious enterocolitis or IgE-mediated anaphylaxis. In order to identify changes specific to acute FPIES, we compared the serum levels of different cytokines in patients with acute FPIES, infectious enterocolitis, and IgE-mediated anaphylaxis. Our results revealed new potential biomarkers for the differential diagnosis of acute FPIES.

Methods

Participants

Seven patients with acute FPIES who were referred to Yamaguchi University Hospital, Mine City Hospital, or Oita University Hospital between March 2008 and September 2019 were enrolled in our study (**Table 1**). The patients were diagnosed according to the criteria for acute FPIES.[6] An OFC test was performed in three patients for diagnosis. The other four patients, in whom OFC tests were not performed, were diagnosed based on episodes fulfilling the criteria for acute FPIES after the ingestion of offensive food. Four patients presented cow milk (CM)-induced FPIES, two patients presented egg yolk-induced FPIES, and one presented wheat-induced FPIES. CM-, egg yolk-, or wheat-specific IgE (sIgE) were absent in all patients. There was one low birth weight and preterm infant. In the FPIES-resolved patient, the condition was induced by egg yolk.

Six patients with infectious enterocolitis [median age, 15 months; range, 3 months to 3 years (36 months)] and nine patients with IgE-mediated food allergy and anaphylaxis [median age, 60 months; range, 8 months to 8 years (99 months)] were enrolled. The infectious enterocolitis group included patients with abdominal symptoms (*e.g.* , vomiting, diarrhea, and bloody stool). Pathogens were identified by stool examination in all patients; norovirus and rotavirus were detected in 2 and 4 patients, respectively. Diagnosis of anaphylaxis was based on the Japanese Guidelines for Food Allergy 2014.[8] Milk, egg, wheat, and buckwheat were the causative food in four, three, one, and one patient with IgE-mediated anaphylaxis, respectively. Informed consent was obtained from the parents and/or patients. The protocol was approved by the Institutional Review Board of Yamaguchi University Hospital (H28- 027).

Blood sample collection

Blood samples from FPIES patients were collected at symptomatic and asymptomatic stages. Samples were collected when the four patients not undergoing the OFC test presented to the hospital due to vomiting within 3 hours after onset or vomited after the intake of causative foods during hospitalization. For the three patients undergoing OFC, samples were collected just after vomiting during the test. The asymptotic

samples were collected from patients without any symptoms such as vomiting or bloody stool. For the evaluation of the cytokine profile, samples were collected at several time points (Patient No. 6: before OFC and 3, 6, and 24 hours after the beginning of OFC; Patient No. 7: before OFC and 0.5, 1, 1.5, 2, and 48 hours after the beginning of OFC; FPIES-resolved control: before OFC and 3, 6, and 24 hours after the beginning of OFC). In the OFC of Patient No. 3, samples were collected only immediately after vomiting. The FPIES-resolved control is an 11-month-old boy whose symptoms were induced by egg yolk.

The blood samples of patients with infectious enterocolitis and anaphylaxis were collected at the time of presentation to the emergency department of the hospital. Samples were centrifuged at 1,400 × $g$for 5 min, and serum was collected and stored at -25 °C.

Cytokine assays

The serum concentrations of the cytokines IL-2, IL-4, IL-6, IL-10, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, were measured using the Cytometric Bead Array Kit (BD Biosciences, San Diego, CA, USA). The serum concentration of IL-8 was measured using Human CXCL8/IL-8 Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). The lower detection limits for IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α, were 2.6, 2.6, 3.0, 3.5, 2.8, 7.1, and 2.8 pg/mL, respectively.

Other laboratory examinations

CM- and egg yolk-sIgE levels were measured using the Fluorescence Enzyme Immunoassay (Special Reference Laboratories, Inc., Tokyo, Japan).

Statistical Analysis

The comparison of serum cytokine levels among acute FPIES, enterocolitis, and IgE-mediated anaphylaxis patient groups was analyzed by Mann-Whitney U test. The serum levels of IL-2 and IL-10 were compared by Student's t -test in samples obtained in symptomatic and asymptomatic stages. Analyses and calculations were performed using JMP® software (SAS Institute Inc., Cary, NC, USA).

Results

Serum cytokine levels in acute FPIES, infectious enterocolitis, and anaphylaxis patients

We compared serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis. Both serum IL-2 and IL-10 levels were significantly higher in patients with acute FPIES than in patients from the other two groups (IL-2: FPIES vs. enterocolitis, $p = 0.003$; FPIES vs. anaphylaxis, $p< 0.001$. IL-10: FPIES vs. enterocolitis, $p = 0.02$; FPIES vs. anaphylaxis, $p = 0.003$; **Figures 1A and 1B, Table 2**). Serum IL-8 level was significantly higher in the acute FPIES group compared with that in the enterocolitis group, but not with that in the anaphylaxis group (acute FPIES vs. enterocolitis, $p = 0.008$; vs. anaphylaxis, $p = 0.24$). Serum IFN-γ level was found to be significantly increased in patients with acute FPIES compared with that in the anaphylaxis group (IFN-γ: FPIES vs. anaphylaxis, $p = 0.04$; **Table 3**). However, the IFN-γ elevation was not as prominent as those detected for IL-2 and IL-10. There were no significant differences in the serum levels of IL-4, -6, and TNF-α among three groups.

Cytokine dynamics in FPIES during acute phase and stable status

To verify whether the elevation in serum IL-2 and IL-10 was specific to the symptomatic period, we compared the levels of the two cytokines in symptomatic and asymptomatic stages in samples from FPIES patients (**Figure 2**). The results showed that the serum levels of both cytokines were significantly higher in the symptomatic than in the asymptomatic period (IL-2 median values: 218.7 pg/mL vs. <2.6 pg/mL, $p = 0.002$; IL-10 median values: 31.8 pg/mL vs. 5.2 pg/mL, $p < 0.001$). Thus, serum IL-2 and IL-10 returned to normal levels when the symptoms disappeared.

Alterations in serum levels of IL-2 and IL-10 during OFC

Next, we evaluated the alterations of serum IL-2 and IL-10 levels during the OFC test in patients No. 6 and No. 7 (**Figures 3A and 3B**). The patients were challenged with causative foods and showed vomiting 3 and 2 hours later, respectively. Notably, a marked increase in the serum levels of IL-2 and IL-10 were observed immediately after vomiting. In patient No. 5, both IL-2 and IL-10 levels were higher at the time of vomiting than 6 hours after the beginning of the OFC (**Figure 3C**). In contrast, no elevation was observed in the two cytokine levels before the OFC test and 24 hours after the beginning of the OFC test. In contrast, this increase was not clearly observed in the FPIES-resolved subject.

Discussion

In this study, we sought to identify potential biomarkers to differentiate acute FPIES from mimicking diseases also associated with vomiting, such as enterocolitis or IgE-mediated anaphylaxis. Our study showed that the serum levels of IL-2 and IL-10 were both prominently elevated in patients with acute FPIES compared with those with enterocolitis or IgE-mediated anaphylaxis. Serum IL-2 and IL-10 increases were only detected in symptomatic FPIES patients. These increases were also confirmed in the symptomatic phase by the OFC test. The serum levels of these two cytokines were previously reported to be elevated in OFC-positive FPIES patients,[4, 5] which is in line with our results.

Several studies on oral immunotherapy have demonstrated that IL-10 levels increase in the course of tolerance acquisition, suggesting a role of this cytokine in the induction of oral tolerance.[5,9] Caubet et al.[5]reported increases in the serum levels of IL-10 in OFC-positive cases, whereas this increase was not observable in the supernatants of casein-stimulated peripheral blood mononuclear cells skewing toward T cells obtained from FPIES patients positive for milk OFC. This suggests that, in patients with active FPIES, since T cells have a reduced capacity to secrete IL-10, other cell populations such as monocytes produce compensatory IL-10 in response to antigen exposure. It has been reported that the activation of innate immune cells is involved in the pathogenesis of acute FPIES.[10] Thus, the increase in serum IL-10 levels that we observed in symptomatic patients could be related to the activation of innate immune cells such as monocytes.

IL-2 is a representative helper T (Th) 1 cytokine, produced by naive and Th cells, that activates T cells, B cells, and monocytes/macrophages.[11] Our finding that serum IL-2 was elevated in patients with acute FPIES was consistent with the results of Kimura et al.[4], who reported that IL-2 elevation contributes to the activation of Th1 cells, which in turn plays a significant role in FPIES pathophysiology. Several lines of evidence suggest a key role of T cells in the secretion of pro-inflammatory cytokines that have an impact on intestinal permeability.[12-16] On the other hand, the role of T cells has been questioned by some studies.[5,10,17,18]It has been reported that IL-2 signaling might be required for maintaining the homeostasis of regulatory T cells.[19]Zhou et al.[20] recently reported that innate lymphoid cell (ILC) 3-derived, but not T cell-derived, IL-2 is essential for maintaining regulatory T cell immunological homeostasis throughout the gastrointestinal tract. This suggests that increased IL-2 serum levels observed in the very early phase of onset in acute FPIES patients could reflect increased IL-2 production promoted by other mechanisms such as ILC3 mediation in the gastrointestinal tract, although the pathophysiological role of IL-2 remains unknown at the mechanistic level.

Some studies have reported elevated serum IL-8 levels in symptomatic acute FPIES patients.[4,5] The serum level of IL-8 was significantly elevated in the acute FPIES group compared with the enterocolitis group. However, there were no significant differences between patients with acute FPIES and IgE-mediated anaphylaxis, suggesting that IL-8 was not a useful biomarker to differentiate acute FPIES from mimicking diseases.

It has been reported that serum IL-2 and IL-10 levels are elevated in severe anaphylactic reactions and correlate with the severity.[21] Furthermore, Jiang et al.[22] and Newman et al.[23] reported cytokine profiles in children with rotavirus infection and patients with norovirus and observed a significant increase in the IL-2 and IL-10 levels compared to that in the corresponding healthy control group or individuals before illness onset. The above-reported increases in IL-2 and IL-10 levels were not as high as those in our acute FPIES patients. Because the half-life of serum IL-2 is very short (6.9 min)[24], the timing of sample collection is very important to detect higher levels of serum IL-2.

Lee et al.[7] also sought features distinctive of acute FPIES by comparing patients who presented to an emergency department with FPIES symptoms and subjects with enterocolitis or sepsis. In their study, the laboratory data characteristic of acute FPIES included less pronounced CRP elevation, leukocytosis, lymphocytosis, and thrombocytosis; and elevated albumin/globulin ratio. Thus, we compared CRP as well as white blood cell, lymphocyte, and platelet counts in patients with acute FPIES, enterocolitis, and IgE-mediated anaphylaxis, and found no significant differences in these parameters among the three groups (data not shown). This discrepancy might reflect differences in the study design and timing of sample collection. Notably, in the study by Lee et al.[7], acute FPIES was determined retrospectively based on the medical record database of an emergency department, and the schedule of sample collection was not specified. In contrast, in the present study, the diagnoses were made in suspected cases by well-trained pediatric allergists based on recent international consensus guidelines[3], and the blood samples were all obtained within 3 h after the onset of vomiting.

In this study, we suggest that serum IL-2 and IL-10 may be suitable biomarkers for the diagnosis of acute FPIES. Acute FPIES can cause lethal hypovolemic shock, and thus, the elimination of offensive foods is crucial.[25] However, causative foods may be reintroduced to patients unless acute FPIES is diagnosed by reliable biomarkers. We propose serum IL-2 and IL-10 as a convenient biomarker to differentiate acute FPIES from other mimicking diseases, thus avoiding the risk of lethal shock due to future accidental ingestion of the offending foods by the patients.

There are some limitations in this study. First, it is a pilot study conducted with a small sample size in a single center. Multicenter studies from different regions are warranted to confirm the diagnostic accuracy of serum IL-2 and IL-10 measurements for discrimination between acute FPIES and mimicking diseases that are also associated with vomiting. Second, we only considered infectious enterocolitis and IgE-mediated anaphylaxis as non-FPIES conditions. Since there are many other acute FPIES-mimicking diseases[2], further analyses need to include other conditions such as necrotizing enterocolitis, sepsis, and lactose intolerance. Third, as we did not use age-matched normal subjects, we have not adjusted the cytokine levels by age.

To our knowledge, this is the first report to identify potential biomarkers capable of discriminating between acute FPIES and other diseases and, therefore, suitable for differential diagnosis. The measurement of serum IL-2 and IL-10 levels may substantially improve the diagnosis of acute FPIES, which now mainly relies on clinical manifestations.

Table 1 Clinical features of patients with acute FPIES	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**	**Table 1 Clinical features of patients with acute FPIES**
No.	**Gender**	**Gestational age (week)**	**Birth weight (g)**	**Age at episode (month)**	**Causative food**	**Symptoms**	**Specific IgE (U_A/mL)**
1	male	36	2,802	2	cow's milk	V, B	< 0.10
2	male	39	3,036	8	egg yolk	V	< 0.10
3	female	38	3,248	9	cow's milk	V	< 0.10
4	female	37	2,689	12	egg yolk	V	< 0.10
5	female	38	2,840	14	cow's milk	V, B, D	< 0.10
6	male	31	1,714	32	wheat	V, F	<0.10
7	male	38	3,244	48	cow's milk	V	< 0.34

B, bloody stool; D, diarrhea; F, fever; FPIES, food protein-induced enterocolitis syndrome; V, vomiting.

Table 2 Serum cytokine profile in patients with acute FPIES	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**	**Table 2 Serum cytokine profile in patients with acute FPIES**
No.	**Age at episode (month)**	**IL-2 (pg/mL)**	**IL-4 (pg/mL)**	**IL-6 (pg/mL)**	**IL-8 (pg/mL)**	**IL-10 (pg/mL)**	**IΦN-γ (πγ/μΛ)**	**TNΦ- (πγ/μ**
1	2	16.1	< 2.6	6.7	192.5	105.2	< 7.1	< 2.8
2	8	172.8	5.3	6.9	311.7	10.9	< 7.1	< 2.8
3	9	335.3	4.7	29.8	1,454.6	13.5	19.6	< 2.8
4	12	25.6	< 2.6	18.5	759.5	59.5	< 7.1	3.0
5	14	471.1	< 2.6	237.5	2,372.7	26.3	41.0	3.3
6	32	1,103.9	< 2.6	22.1	980.7	31.8	< 7.1	8.4
7	48	218.7	5.1	28.4	1263.0	83.1	17.1	7.6

FPIES, food protein-induced enterocolitis syndrome; IL, interleukin.

Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**
	Acute FPIES	**Infectious enterocolitis**	**Anaphylaxis**	***p* value**	***p* value**
Cytokine				**Acute FPIES vs. Infectious enterocolitis**	**Acute FPIES vs. Anaphylaxis**
IL-4 (pg/mL)	< 2.6 (< 2.6-5.3)	< 2.6	< 2.6 (< 2.6-4.3)	0.08	0.13
IL-6 (pg/mL)	22.1 (6.7-237.5)	11.0 (3.7-25.7)	22.2 (3.4-724.0)	0.92	0.92
IL-8 (pg/mL)	980.7 (192.5-2,372.7)	44.3 (11.1-621.3)	147.7 (< 3.5-11,212.8)	0.005*	0.24

Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**	**Table 3 Serum cytokine levels in patients with acute FPIES, infectious enterocolitis, and anaphylaxis (except IL-2 and IL-10)**
IΦN-γ (πγ/μΛ)	< 7.1 (< 7.1-41.0)	< 7.1 (< 7.1-11.2)	< 7.1	0.57	0.04*
TNΦ-α (πγ/μΛ)	3.0 (< 2.8-8.4)	< 2.8 (< 2.8-3.9)	< 2.8 (< 2.8-3.7)	0.14	0.61

FPIES, food protein-induced enterocolitis syndrome; IL, interleukin; $*p < 0.05$.

References

1. Järvinen K, Nowak-Wegrzyn A. Food protein-induced enterocolitis syndrome: current management strategies. J Allergy Clin Immunol Pract 2013;1:317-322.
2. Nowak-Wegrzyn A, Jarocka-Cyrta E, Moschione Castro A. Food Protein-Induced Enterocolitis Syndrome. J Investig Allergol Clin Immunol 2017;27:1-18.
3. Morita H, Nomura I, Orihara K, Yoshida K, Akasawa A, Tachimoto H, Ohtsuka Y, Namai Y, Futamura M, Shoda T, Matsuda A, Kamemura N, Kido H, Takahashi T, Ohya Y, Saito H, Matsumoto K. Antigen-specific T-cell responses in patients with non-IgE-mediated gastrointestinal food allergy are predominantly skewed to T(H)2. J Allergy Clin Immunol 2013;131:590-592.
4. Kimura M, Ito Y, Shimomura M, Morishita H, Meguro T, Adachi Y, Seto S. Cytokine profile after oral food challenge in infants with food protein-induced enterocolitis syndrome. Allergol Int 2017;66:452-457.
5. Caubet JC, Bencharitiwong R, Ross A, Sampson HA, Berin MC, Nowak-Wegrzyn A. Humoral and cellular responses to casein in patients with food protein-induced enterocolitis to cow's milk. J Allergy Clin Immunol 2017;139:572-583.
6. Nowak-Wegrzyn A, Chehade M, Groetch ME, Spergel JM, Wood RA, Allen K, Atkins D, Bahna S, Barad AV, Berin C, Brown Whitehorn T, Burks AW, Caubet JC, Cianferoni A, Conte M, Davis C, Fiocchi A, Grimshaw K, Gupta R, Hofmeister B, Hwang JB, Katz Y, Konstantinou GN, Leonard SA, Lightdale J, McGhee S, Mehr S, Sopo SM, Monti G, Muraro A, Noel SK, Nomura I, Noone S, Sampson HA, Schultz F, Sicherer SH, Thompson CC, Turner PJ, Venter C, Westcott-Chavez AA, Greenhawt M. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary - Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol 2017; 139:1111-1126.
7. Lee E, Barnes EH, Mehr S, Campbell DE. Differentiating Acute Food Protein-Induced Enterocolitis Syndrome From Its Mimics: A Comparison of Clinical Features and Routine Laboratory Biomarkers. J Allergy Clin Immunol Pract 2019;7:471-478.
8. Urisu A, Ebisawa M, Ito K, Aihara Y, Ito S, Mayumi M, Kohno Y, Kondo N. Japanese Guideline for Food Allergy 2014. Allergol Int 2014;63:399-419.
9. Battaglia M, Gianfrani C, Gregori S, Roncarolo MG. IL-10-producing T regulatory type 1 cells and

oral tolerance. Ann N Y Acad Sci 2004;1029:142-153.
10. Goswami R, Blazquez AB, Kosoy R, Rahman A, Nowak-Wegrzyn A, Berin MC. Systemic innate immune activation in food protein-induced enterocolitis syndrome. J Allergy Clin Immunol 2017;139:1885-1896.
11. Arenas-Ramirez N, Woytschak J, Boyman O. Interleukin-2: Biology, Design and Application. Trends Immunol 2015;36:763-777.
12. Caubet JC, Szajewska H, Shamir R, Nowak-Wegrzyn A. Non-IgE-mediated gastrointestinal food allergies in children. Pediatr Allergy Immunol 2017;28:6-17.
13. Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. Allergy 2005;60:912-919.
14. Van Sickle GJ, Powell GK, McDonald PJ, Goldblum RM. Milk- and soy protein -induced enterocolitis: evidence for lymphocyte sensitization to specific food proteins. Gastroenterology 1985;88:1915-1921.
15. Hoffman KM, Ho DG, Sampson HA. Evaluation of the usefulness of lymphocyte proliferation assays in the diagnosis of allergy to cow's milk. J Allergy Clin Immunol 1997;99:360-366.
16. Mori F, Barni S, Cianferoni A, Pucci N, de Martino M, Novembre E. Cytokine expression in CD3+ cells in an infant with food protein-induced enterocolitis syndrome (FPIES): case report. Clin Dev Immunol 2009;2009:679381.
17. Ruffner MA, Ruymann K, Barni S, Cianferoni A, Brown-Whitehorn T, Spergel JM. Food protein-induced enterocolitis syndrome: insights from review of a large referral population. J Allergy Clin Immunol Pract 2013;1:343-349.
18. Järvinen KM, Caubet JC, Sickles L, Ford LS, Sampson HA, Nowak-Wegrzyn A. Poor utility of atopy patch test in predicting tolerance development in food protein-induced enterocolitis syndrome. Ann Allergy Asthma Immunol 2012;109:221-222.
19. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol 2005;6:1142-1151.
20. Zhou L, Chu C, Teng F, Bessman NJ, Goc J, Santosa EK, Putzel GG, Kabata H, Kelsen JR, Baldassano RN, Shah MA, Sockolow RE, Vivier E, Eberl G, Smith KA, Sonnenberg GF. Innate lymphoid cells support regulatory T cells in the intestine through interleukin-2. Nature 2019;568:405-409.
21. Stone SF, Cotterell C, Isbister GK, Holdgate A, Brown SG, Emergency Department Anaphylaxis Investigators. Elevated Serum Cytokines During Human Anaphylaxis: Identification of Potential Mediators of Acute Allergic Reactions. J Allergy Clin Immunol 2009;124:786-792.e4.
22. Jiang B, Snipes-Magaldi L, Dennehy P, Keyserling H, Holman RC, Bresee J, Gentsch J, Glass RI. Cytokines as Mediators for or Effectors Against Rotavirus Disease in Children. Clin Diagn Lab Immunol 2003;10:995-1001.
23. Newman KL, Moe CL, Kirby AE, Flanders WD, Parkos CA, Leon JS. Norovirus in symptomatic and asymptomatic individuals: cytokines and viral shedding. Clin Exp Immunol 2016;184:347-357.
24. Lotze MT, Matory YL, Ettinghausen SE, Rayner AA, Sharrow SO, Seipp CA, Custer MC, Rosenberg SA.. In Vivo Administration of Purified Human Interleukin 2. II. Half Life, Immunologic Effects, and Expansion of Peripheral Lymphoid Cells in Vivo With Recombinant IL 2. J Immunol 1985;135:2865-2875.
25. Miceli Sopo S, Dello Iacono I, Greco M, Monti G. Clinical management of food protein-induced enterocolitis syndrome. Curr Opin Allergy Clin Immunol 2014;14:240-245.

Figure legends

Figure 1. Serum levels of IL-2 (**A**) and IL-10 (**B**) in acute FPIES, infectious enterocolitis, and anaphylaxis. IL, interleukin.

Figure 2. Cytokine dynamics in patients with acute FPIES in symptomatic and asymptomatic phase. The comparison of cytokine levels during acute episodes and in stable status is shown. Each long bar indicates the median value (IL-2: symptomatic and asymptomatic phase; 218.7 pg/ml vs. < 2.6 pg/ml, IL-10: symptomatic and asymptomatic phase; 31.8 pg/ml vs. 5.2 pg/ml) and each short bar indicates the

mean value (IL-2: symptomatic and asymptomatic phase; 334.8 pg/ml vs. 2.8 pg/ml, IL-10: symptomatic and asymptomatic phase; 47.2 pg/ml vs. 6.5 pg/ml). FPIES, food protein-induced enterocolitis syndrome; IL, interleukin.

Figure 3. Time course of cytokines in FPIES patient No. 6 (**A**), patient No. 7 (**B**), and FPIES resolved patient (control) (**C**). FPIES, food protein-induced enterocolitis syndrome.

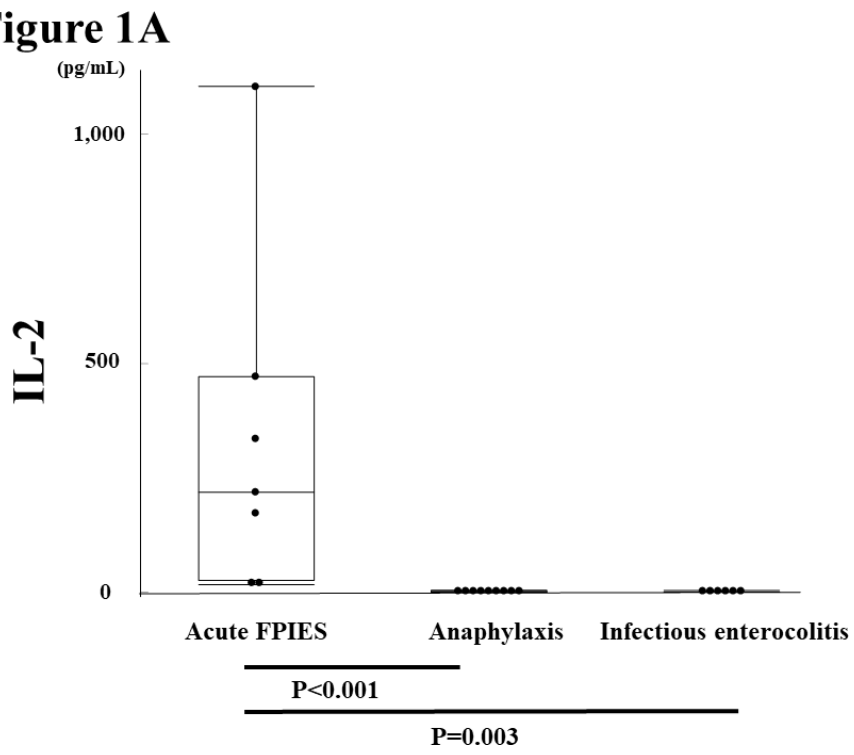

Figure 1B

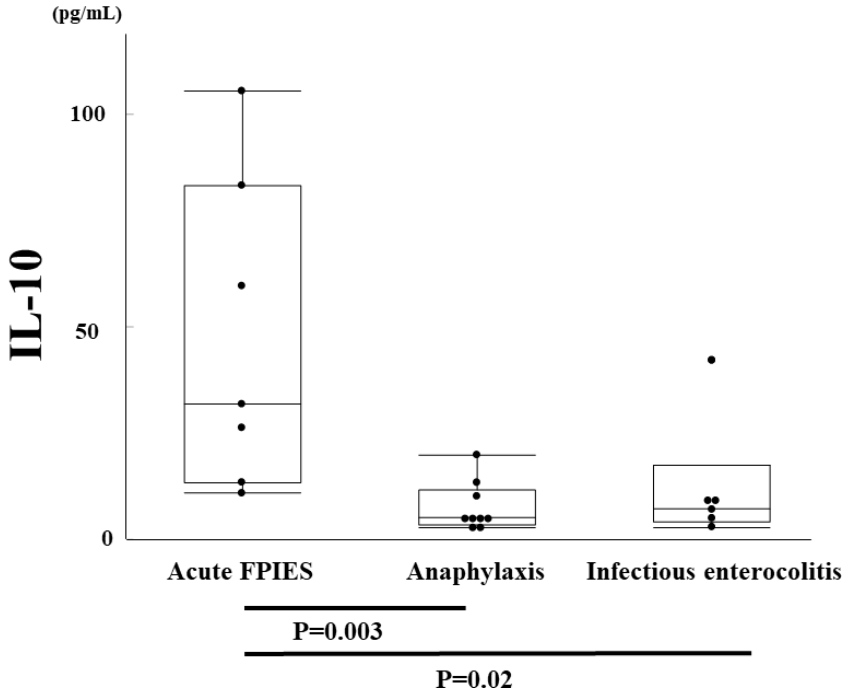

Figure 2

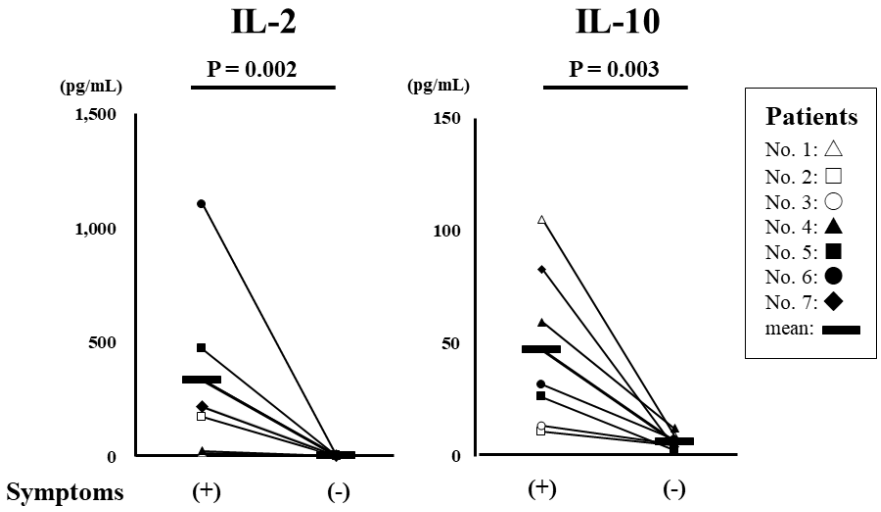

Figure 3A

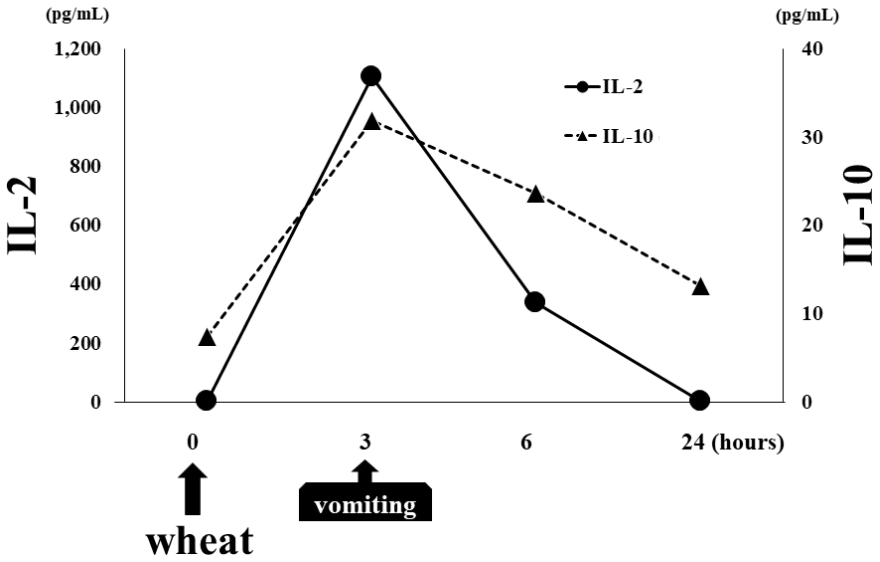

Figure 3B

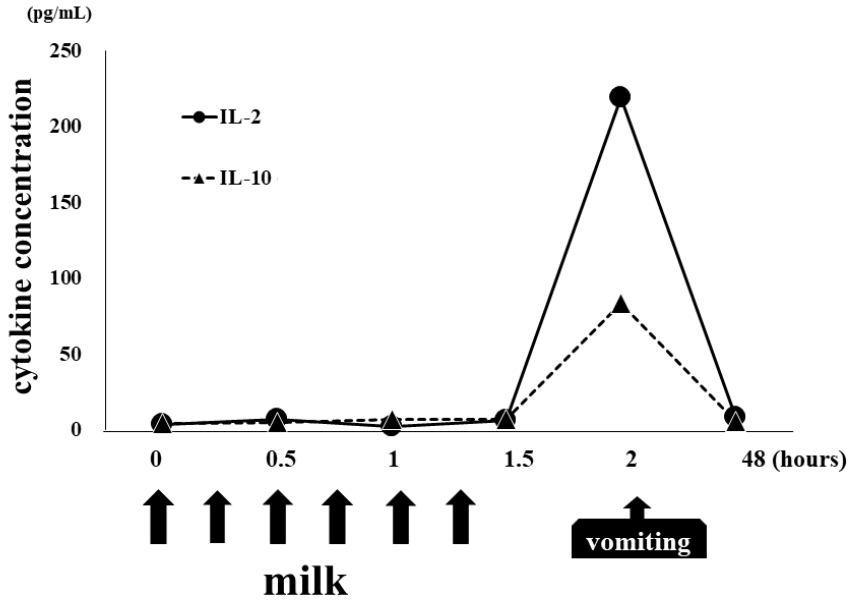

Figure 3C